Osteoarthritis and Cartilage (2008) **16,** 660–666

doi:10.1016/j.joca.2007.10.002

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Relationships between biochemical markers of bone and cartilage degradation with radiological progression in patients with knee osteoarthritis receiving risedronate: the Knee Osteoarthritis Structural Arthritis randomized clinical trial[1]

P. Garnero Ph.D.†‡*, W. S. Aronstein M.S.§, S. B. Cohen M.D.‖, P. G. Conaghan M.S.¶, G. A. Cline§, C. Christiansen M.D.#, J. F. Beary M.D.§, J. M. Meyer Ph.D.§ and C. O. Bingham III M.D.††

† *INSERM Research Unit 664, Lyon, France*
‡ *Molecular Markers, Synarc, Lyon, France*
§ *Procter & Gamble Pharmaceuticals, Mason, OH, USA*
‖ *Department of Radiology, St. Paul Medical Center, Dallas, USA*
¶ *Academic Unit of Musculoskeletal Disease, University of Leeds, UK*
# *Center for Clinical and Basic Research, Copenhagen, Denmark*
†† *Johns Hopkins University, Baltimore, MD, USA*

Summary

Objective: To investigate whether early changes in biochemical markers of bone (NTX-I) and cartilage (CTX-II [C-terminal crosslinking telopeptide of type II collagen]) degradation are associated with radiological progression in patients with knee osteoarthritis (OA) receiving risedronate.

Design: Two thousand four hundred and eighty three patients with medial compartment knee OA were randomized in two 24-month studies in North America (NA) and European Union (EU). Studies evaluated risedronate 5 mg/day, 35 mg/week (EU), 50 mg/week (NA), and 15 mg/day (NA and EU), compared to placebo in reducing signs and symptoms and in slowing radiographic progression. One thousand eight hundred and eighty five patients from the pooled EU and NA studies with available NTX-I/CTX-II at both baseline and 6 months and radiographs at baseline and at 24 months were analyzed.

Results: Risedronate produced a dose-dependent reduction of NTX-I and CTX-II observed at 6 months which continued up to 24 months. Patients who had CTX-II levels returned to low levels (<150 ng/mmol creatinine) at 6 months had a lower risk of radiographic progression at 24 months than patients whose CTX-II levels were increased both at baseline and 6 months [odds-ratio (95% confidence interval): 0.57 (0.39–0.85) after adjustment for demographics and joint space width]. The lowest risk of progression was observed in patients who had low CTX-II levels both at baseline and at 6 months [odds-ratio 0.36 (0.21–0.63)]. No significant association between NTX-I levels and radiological progression was observed.

Conclusion: CTX-II decreased with risedronate in patients with knee OA and levels reached after 6 months were associated with radiological progression at 24 months. Monitoring a marker of cartilage degradation 6 months after initiating treatment may be instructive in identifying patients with low progression.

Key words: Osteoarthritis, Biochemical marker, Type II collagen, Bisphosphonate.

Introduction

Osteoarthritis (OA) is a prevalent disease that particularly affects weight-bearing joints such as knees and hips[1]. Current therapies for OA are mainly targeted toward providing symptom relief[2], and only a few studies have reported efficacy of drugs on modifying disease progression including preservation of radiological joint structure[3–6]. Although the major structural feature of OA is cartilage degeneration, OA is also characterized by osteophyte formation, synovial activation, and subchondral bone abnormalities.

The participation of subchondral bone alterations in the pathogenesis of OA has initially been suggested by bone scan studies showing that increased tracer uptake is associated with increased risk of radiographic progression in OA of the knee[7]. The role of subchondral bone abnormalities as a major factor in disease progression has also been supported by the use of magnetic resonance imaging (MRI) and biochemical markers of bone turnover[8–10]. Consequently, modulating subchondral bone turnover by anti-resorptive agents such as bisphosphonates has become an attractive approach as a potential structure modifying anti-OA drug (SMOAD) target.

[1]This manuscript was sponsored by a grant from Procter & Gamble.
*Address correspondence and reprint requests to: Dr Patrick Garnero, Ph.D., INSERM Research Unit 664 and Molecular Markers, Synarc, 16 rue de Montbrillant, 69003 Lyon, France. Tel: 33-4-72-68-65-05; Fax: 33-4-72-68-65-08; E-mail: patrick.garnero@synarc.com
Received 10 July 2007; revision accepted 1 October 2007.

Although joint space narrowing (JSN) measured using plain radiography is the primary measure to assess structural progression currently accepted by regulatory agencies in pivotal clinical trials, soluble biochemical markers may be more sensitive in detecting short term changes because they reflect dynamic biological variation[11]. Several markers have been described which differ by tissue specificity and sensitivity in detecting alterations in OA[11,12]. Among these, a single measurement of urinary cross-linked C-telopeptides of type II collagen (CTX-II [C-terminal crosslinking telopeptide of type II collagen]), a marker of cartilage degradation, has been associated with radiological progression in patients with knee and hip OA[13–15]. Retrospective analyses of clinical trials in postmenopausal women have shown that the bisphosphonates alendronate, ibandronate and risedronate administered at approved doses for osteoporosis induced a significant decrease of CTX-II[16]. A dose-dependent reduction of CTX-II was also seen with risedronate given at 5 mg/day and 15 mg/day in a 1 year placebo-controlled study of patients with knee OA[17]. We reported earlier that risedronate did not decrease symptoms or radiological progression compared to placebo over 24 months in two randomized phase III trails[18]. We now present further analysis of data in that study on bone and cartilage markers and their association to radiological progression.

Patients and methods

STUDY DESIGN AND SELECTION OF PATIENTS

Two parallel 24-month double-blind placebo-controlled, multicenter European (EU) and North America (NA) studies were conducted. A detailed description of these studies has recently been published[18]. Briefly, male and female subjects between 40 and 80 years of age and fulfilling the American College of Rheumatology[19] criteria for symptomatic primary knee OA were recruited.

Clinical inclusion criteria were the presence of signal knee pain due to OA on most days during at least 1 month in a 3-month period prior to screening plus at least one of the following: age $>$ 50 years, morning knee stiffness lasting $<$30 min or knee crepitus. All patients then underwent radiography of the knee to confirm the presence of OA using standardized fluoroscopically positioned semiflexed anterioposterior (AP) views as previously described[20]. The radiographic inclusion criteria were a joint space width (JSW) between 2 and 4 mm inclusive in the medial knee tibiofemoral compartment and the presence of at least one osteophyte in the medial or lateral compartments of the tibiofemoral joint. Patients with concomitant presence of lateral JSN could be included in the study, but the medial tibiofemoral compartment has to be narrower than the lateral. If both knees qualified, the signal knee was defined as the knee with smaller JSW. Major exclusion criteria were secondary OA, use of intra-articular hyaluronic acid in the signal knee, knee injury or diagnostic arthroscopy of the signal knee in the 6 months prior to enrollment, history of knee surgery in the signal knee at any time, intra-articular corticosteroids in the 3 months preceding enrollment, non-OA causes of knee pain in the signal knee such as anserine bursitis, fibromyalgia and osteonecrosis, and use of bisphosphonates within 12 months prior to enrollment.

Patients gave written, informed consent. The study was conducted according to the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (GCP), and approved by local ethical committees. It was conducted before the requirement for registration on clinicaltrials.gov.

TREATMENT ASSIGNMENT

The EU study included 1255 patients to receive oral risedronate (5 mg/day, 15 mg/day or 35 mg/week) or placebo. The NA trial included 1242 patients to receive oral risedronate (5 mg/day, 15 mg/day or 50 mg/week) or placebo. Patients were instructed to take their study medication with at least 120 mL of water, 30 min before breakfast, or, if the medication was taken later in the day, at least 2 h prior to or following food ingestion and at least 30 min before bedtime.

STRUCTURE OUTCOME MEASURES

The signal knee JSW was evaluated at baseline, 1 and 2 years at the narrowest point in the medial tibiofemoral compartment using standardized AP radiographs, aided by fluoroscopy, and by attaching a metal sphere to the fibula head to correct magnification effects according to the procedure of Buckland-Wright[20,21]. Each knee was flexed until the tibial plateau was horizontal relative to the floor, parallel to the central X-ray beam and perpendicular to the X-ray film. The center of the joint, defined by the joint space, was aligned with the center of the X-ray beam with the aid of the tube's positioning light. The precise knee position was obtained visually with the aid of fluoroscopy. With the heel fixed, the foot was internally or externally rotated until the tibial spines appeared centrally placed relative to the femoral notch; then the knee was flexed to achieve superimposition ($\pm$1 mm) of the anterior and posterior margins of the medial tibial plateau. Minimum medial compartment JSW was measured with a semi-automated computerized method. The test/re-test standard deviation (SD) of the difference between radiographs taken 2 days apart was approximately 0.2 mm based upon repeated measurements in 199 subjects[21]. Based on this reproducibility data, clinically meaningful disease progression was defined *a priori* as a JSN of $\geq$0.6 mm from baseline which corresponded to approximately three times the SD of the X-ray measurement method for joint space.

MARKERS OF BONE AND CARTILAGE RESORPTION

Early morning fasting urine samples were collected at baseline, months 6, 12 and 24 for measurement of urinary N-terminal crosslinking telopeptide of type I collagen (NTX-I, Osteomark; OrthoClinical Diagnostics, Rochester, NY)[22] – a marker of bone resorption – and urinary CTX-II (Cartilaps, Nordic Bioscience, Herlev, Denmark)[23]. Intra- and inter-assay coefficients of variation were lower than 10% for both markers. Urinary NTX-I and CTX-II were corrected for urinary creatinine concentration measured by a standard colorimetric assay.

STATISTICAL ANALYSIS

The analysis was performed on all randomized patients who received at least one dose of study medication and who had baseline and 6 months marker data available. All statistical analyses were performed using a two-sided test with a type I error rate of 0.05. This *post hoc* analysis was based on the relationships between baseline and 6-month levels of biochemical markers and radiological progression at 24 months, data that were available on a total of 1885 patients for the pooled EU and NA studies. We also analyzed percent changes of NTX-I and CTX-II at 12 and 24 months on a total of 1825 and 1920 patients, respectively. Distribution free confidence limits for the medians were constructed based on the methods described by Hahn and Meeker[24]. Significance of percent changes from baseline and placebo in NTX-I and CTX-II was assessed by analysis of variance (ANOVA).

We constructed a logistic regression model including all known predictors of progression measured in this cohort. Baseline predictors included body mass index (BMI), gender, Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) pain score, presence/absence of hip OA, knee crepitus and JSW. Additionally, levels of NTX-I or CTX-II at baseline and changes at 6 months were added into the model as a continuous variable. We also entered baseline and 6-month levels of CTX-II as a categorical variable (high vs low, see Table II). Two cut-off values of CTX-II to differentiate high vs low cartilage turnover were used: (1) the mean level of healthy individuals with no symptomatic OA[25,26]. This cut-off was previously used and shown to be associated with radiological progression in patients with rheumatoid arthritis (RA) treated with a disease modifying therapy[27] and (2) the optimal values at baseline and at 6 months to discriminate radiological progressors and non-progressors at 24 months identified by receiver operating curve (ROC) analysis. These values of 297 and 224 ng/mmol creatinine at baseline and at 6 months, respectively, are close to the cut-point of 251 ng/mmol creatinine identified previously to be associated with increased risk of radiological knee OA progression in the largest epidemiological cohort that has investigated CTX-II[14].

Results

PATIENTS

The total numbers of patients in the intention to treat (ITT) population were 1251 and 1232 for the EU and NA studies, respectively[18]. European subjects were 3 years older than North Americans (mean $\pm$ SE: 63.6 $\pm$ 0.23 years vs 60.5 $\pm$ 0.25 years, $P < 0.0001$) and a larger proportion of them were female (79 vs 61%, $P < 0.0001$). A larger proportion of NA postmenopausal women was on hormone replacement therapy (44 vs 12%, $P < 0.0001$). The WOMAC total score and subscales were higher in European compared to North American patients (mean $\pm$ SE total

WOMAC score: 45.8 ± 0.59 vs 40.2 ± 0.65, $P < 0.0001$). BMI was slightly higher ($P < 0.0001$) in NA patients than in EU patients (mean ± SE: 30.3 ± 0.14 vs 29.4 ± 0.12), whereas the minimum JSW was not different (mean ± SE: 2.971 ± 0.017 vs 2.973 ± 0.017 mm for EU and NA populations, respectively, $P = 0.94$). Consistent with the higher age and the larger proportion of postmenopausal women, the mean values of NTX-I (mean ± SE: 48.1 ± 0.77 vs 37.5 ± 0.66 nmol/mmol creatinine, $P < 0.001$ in EU and NA studies respectively) and CTX-II (366.6 ± 7.1 vs 284.9 ± 6.7 ng/mmol creatinine, $P < 0.001$) were also higher in the EU than in the NA patients. As previously shown[18], in both studies, the different treatment groups were well balanced for demographics, WOMAC scores, JSW and NTX-I and CTX-II levels.

Table I shows the baseline characteristics of the 1885 patients (out of the total 2483 subjects from the pooled EU and NA studies) included in this analysis who had data available for baseline and 6-month levels of NTX-I/CTX-II and radiological progression at 24 months and the other 598 patients. Age, BMI and baseline minimum JSW were very similar and not statistically significant different between the two groups. The proportion of patients with knee crepitus and concomitant hip OA was also similar (NS). There was a larger proportion of female patients among subjects included in this analysis (72 vs 65%, $P = 0.0024$) and the WOMAC pain score was slightly lower ($P = 0.0019$) (Table I).

EFFECTS OF RISEDRONATE ON MARKERS OF BONE AND CARTILAGE DEGRADATION

Risedronate induced a dose-dependent decrease of NTX-I and CTX-II [Fig. 1(A) and (B)]. In both EU and NA studies, the decrease of CTX-II already reached a plateau after 6 months of treatment which was sustained over 24 months. In the EU study, at 6 months, 5 mg/day risedronate decreased CTX-II by −16.2 ± 2.8 (median: −25.9%) relative to baseline ($P < 0.05$ vs baseline and placebo). The decrease of CTX-II observed with the equivalent weekly dose of 35 mg/week was of similar magnitude to the 5 mg/day dose [Fig. 1(A)]. The largest dose of 15 mg/day produced the greatest decreases of CTX-II, reaching a mean ± SE of −36.9 ± 3.0 (median: −49.5%) at 6 months [Fig. 1(A)]. Results observed in the NA study were similar, both in terms of pattern and magnitude of changes for equivalent doses to those of the EU trial [Fig. 1(B)].

Table I
Baseline demographics of patients included in the current analysis and the other patients

Parameter	Patients in the study* ($N = 1885$)	Other patients ($N = 598$)
Age (years)	62.0 ± 8.56	62.2 ± 9.17
Female gender, *n* (%)	1357 (72%)	391 (65%)
BMI (kg/m^2)	29.9 ± 4.58	29.8 ± 4.41
WOMAC pain score	39.1 ± 21.92	42.3 ± 21.65
Minimum JSW (mm)	3.0 ± 0.60	2.9 ± 0.57
Patients with knee crepitus, *n* (%)	1559 (83%)	500 (84%)
Patients with OA at the hip, *n* (%)	329 (17%)	92 (15%)

All data are shown as mean ± SD for quantitative variable.
*Patients with available baseline and 6-month NTX-I/CTX-II data and 24-month radiological progression.

RELATIONSHIPS BETWEEN BIOCHEMICAL MARKERS AND RADIOGRAPHIC PROGRESSION

Because there was no statistical difference in radiographic progression analyzed either in continuous or dichotomous variables (joint space loss greater or lower than 0.6 mm) between treatment groups or between the EU and NA cohorts[18], the three risedronate treatment arms from NA and EU were combined with placebo for these analyses.

As previously reported[18], overall 13% of patients had radiographic progression as defined as a joint space loss ≥ 0.6 mm (Table II). When entered in a logistic regression model as a continuous variable, we found that both baseline CTX-II levels ($P = 0.0003$) and the absolute ($P = 0.0049$) or the percent ($P = 0.0063$) change of CTX-II at 6 months from baseline were significantly associated with 24 months radiological progression after adjusting for BMI, gender, WOMAC pain, presence of hip OA, knee crepitus, treatment and baseline JSW for all analyses and baseline CTX-II for the change and percent change analyses. Table II shows the mean radiological joint space loss, the percentage of patients with radiographic progression at 24 months and the relative risk of progression, stratified for baseline and 6 months levels of CTX-II (high vs low). Patients with low levels of CTX-II both at baseline and at 6 months had a lower radiological joint space loss and a lower proportion of them demonstrated a significant radiological progression (JSN ≥ 0.6 mm) after 24 months than patients who started the study with increased CTX-II level and in whom values remained high after 6 months. Patients who started with high CTX-II levels at baseline who returned to low levels at 6 months had also a significantly lower risk of progression than patients with sustained high CTX-II values, although the actual proportion of progressors in the two groups was similar (11 vs 15%) (Table II). When the cut-off values based on ROC were used to define high and low CTX-II, similar results were obtained (data not shown). We found no significant association between NTX-I levels at baseline and 6 months when entered in the logistical model as a continuous or as a categorical variables and radiological progression at 24 months (data not shown).

At baseline, 81% of all patients had high CTX-II values (>150 ng/mmol creatinine). After 6 months, this proportion remains similar in patients receiving placebo (83%), but dose-dependently decreased with increasing doses of risedronate (Fig. 2). Even with the highest dose of risedronate (15 mg/day), still 50% of patients had CTX-II > 150 ng/mmol after 6 months of treatment. Overall 68% of patients had CTX-II values > 150 ng/mmol after 6 months of treatment.

Discussion

In this study we report that daily or weekly oral risedronate produced a dose-dependent decrease of urinary NTX-I and CTX-II which was observed as early as 6 months after initiating risedronate and which was maintained through the 24 months treatment period. More importantly, the baseline and 6 months levels of CTX-II were associated with radiological progression at 24 months, independently of clinical and radiological risk factors. This suggests that the measurement of CTX-II at baseline and after 6 months of follow-up in combination with other known risk factors may be useful to predict disease progression in knee OA.

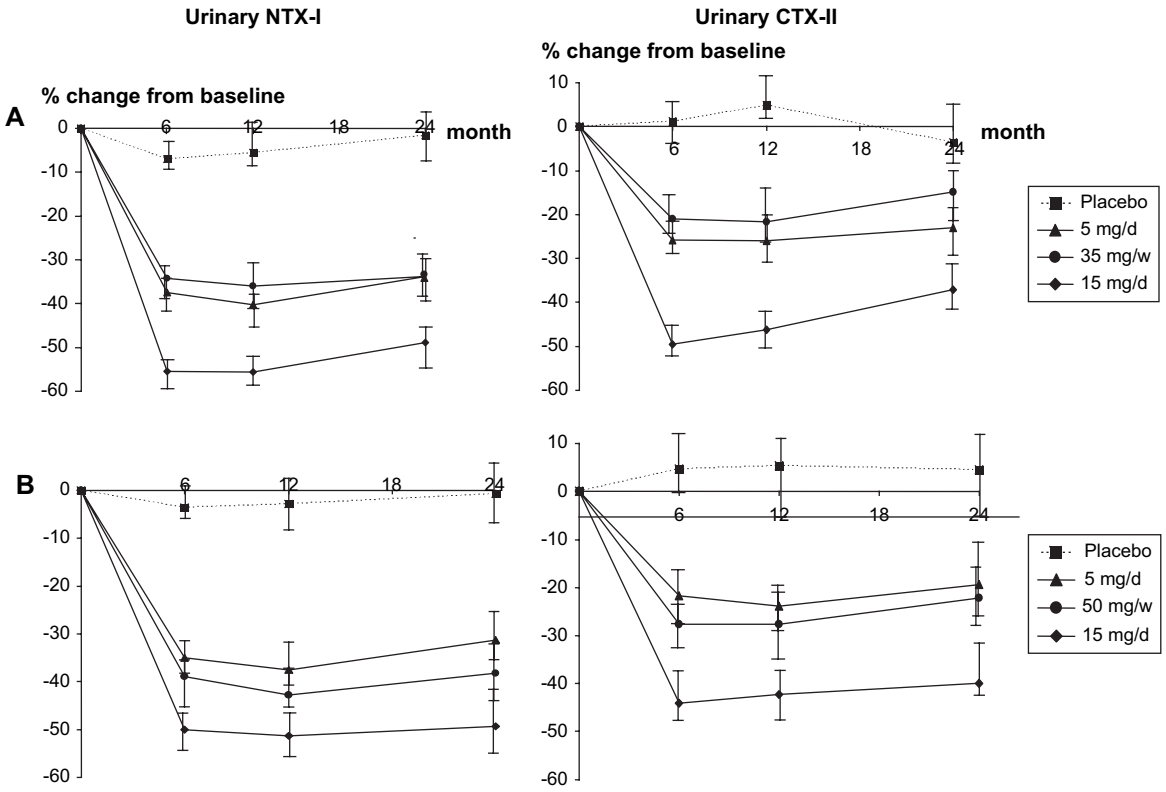

Fig. 1. Changes of NTX-I and CTX-II in patients with knee OA treated with placebo or risedronate. The graphs show the median and the confidence interval limits of the percentage change from baseline of NTX-I (left) and CTX-II (right) in the European (A) or North American (B) study.

We observed a dose-dependent decrease of CTX-II in agreement with the previously reported effects of different bisphosphonates in postmenopausal women with osteoporosis[16]. The assay for CTX-II uses a monoclonal antibody directed against a type II collagen C-telopeptide sequence which is common to various animal species, but specific for type II collagen[23]. *In vitro* animal and human models of cartilage and bone explants have shown that CTX-II is released during degradation of articular cartilage and not of bone matrix[23,28]. In patients with Paget's disease of bone who are characterized by a marked increased of bone resorption, urinary CTX-II is not elevated compared to healthy controls[29].

Immuno-histochemistry of cartilage from ovariectomized rats[30] and patients with knee OA[31] indicates that CTX-II epitope is not only released at the surface of the cartilage, but also at the bone to cartilage interface region within the calcified region which is likely to be preferentially altered by bone anti-catabolic drugs. The decrease of CTX-II we observed with risedronate may thus in part result from indirect effects of the drug on subchondral bone turnover. Pre-clinical studies have shown that risedronate preserves subchondral bone micro-architecture[32]. More recently using fractal analysis of knee radiographs in a subgroup of patients with knee OA included in the current study, it was shown that the doses of 15 mg/day and 50 mg/week of risedronate, but not the lower dose of 5 mg/day, maintain the structural integrity of the cancellous bone of the medial compartment of the proximal tibia[33]. By regulating osteoclast activity, risedronate could also reduce the secretion of yet to be identified pro-catabolic factors which could increase cartilage degradation.

A direct effect of risedronate on cartilage metabolism could also be possible. A single intravenous injection of

Table II
CTX-II at baseline and 6 months for predicting 24-month radiographic progression

CTX-II levels (baseline/6 months*)	Mean ± S.E.M. of change in JSW at 24 months		Radiographic progression† at 24 months	
	Absolute change (mm)	% change from baseline	Progressor (%)	Relative risk (95% CI)‡
High/high ($n = 1152$)	-0.121 ± 0.015	-4.2 ± 0.5	15	1.0 (reference)
High/low ($n = 372$)	-0.088 ± 0.023	-2.9 ± 0.8	11	0.57 (0.39–0.85)
Low/high ($n = 120$)	-0.108 ± 0.039	-4.5 ± 1.5	13	0.77 (0.43–1.36)
Low/low ($n = 241$)	-0.041 ± 0.024	-1.6 ± 0.9	6	0.36 (0.21–0.63)

*Baseline and 6 month cut-off value of CTX-II at 150 nmol/mmol creatinine to separate low and high levels.
†Progression was defined as a JSN $\geq$ 0.6 mm at any post baseline visit.
‡Relative risks were adjusted for BMI, gender, WOMAC pain, hip OA, knee crepitus at baseline, baseline JSW and treatment allocation.

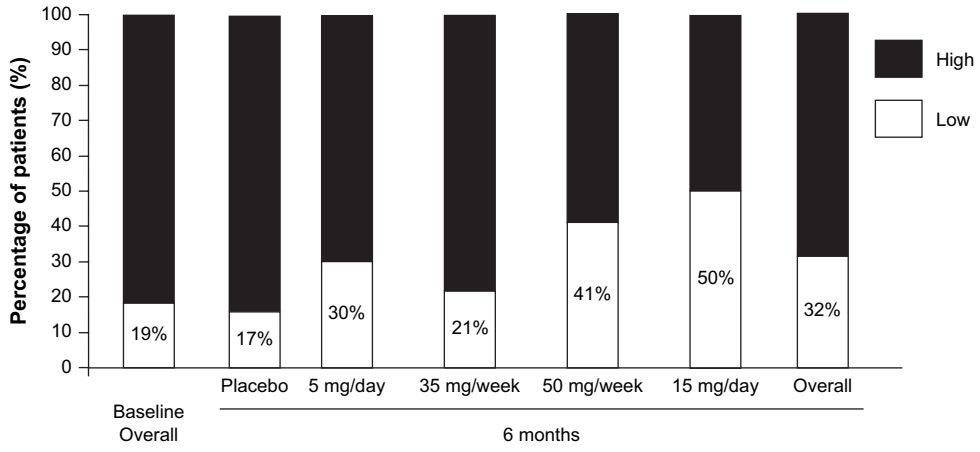

Fig. 2. Cartilage turnover classification at baseline and at 6 months. The bars show the percentage of patients with knee OA with high and low urinary CTX-II levels at baseline and after 6 months of treatment with placebo or different doses of risedronate. Patients were categorized as low (<150 ng/mmol creatinine; white) or high (≥150 ng/mmol creatinine; black) levels of urinary CTX-II.

zoledronic acid produces an acute transient reduction of urinary CTX-II in patients with Paget's disease in contrast to the sustained inhibition of bone resorption as measured by urinary NTX-I[29]. It has been reported that alendronate, inhibits *in vitro* the activity of matrix-metalloprotease 13 (MMP-13) which is one of the key enzymes involved in cartilage degradation in OA[34] and in the release of CTX-II fragments from cartilage[28,30] (unpublished observations). In addition to its direct inhibitory effect on enzyme activity, bisphosphonate could also reduce the secretion of MMPs[35]. In summary, the rapid decrease of urinary CTX-II following risedronate may result from a direct and/or indirect effect of that bisphosphonate on articular and/or calcified cartilage degradation. We can also not exclude the possibility that bone matrix contains very small amounts of type II collagen and thus that inhibition of skeletal turnover by risedronate could contribute to decreased urinary CTX-II levels.

We explored whether monitoring levels of biochemical markers during the course of the study could predict radiological progression over 24 months. For NTX-I, we found no significant predictive value of either baseline or 6-month levels, confirming previous studies in non-bisphosphonate treated patients indicating that bone turnover markers are poorly predictive of radiological progression in OA[11,15]. In contrast, we found associations between 6-month levels of CTX-II and 24-month radiological progression, these associations being significant after adjustment for the other clinical and radiological variables shown to be associated with progression. The value of baseline CTX-II level to predict radiological progression has been reported in patients with knee or hip OA who were not taking bisphosphonates[13–15]. Our study is, however, the first one demonstrating a relationship between radiological progression and longitudinal changes of CTX-II after 6 months in patients receiving risedronate. When entered as a continuous variable we found that both baseline levels and changes (absolute change or percentage change) from baseline at 6 months of CTX-II were associated with radiological progression. In further analyses, however, we have focused on the absolute levels of CTX-II at 6 months as a predictor of radiological progression and not the change from baseline both for biological and statistical principles. Indeed, a treatment whose aim is to prevent joint damage should bring the level of markers reflecting that process to the one of healthy non-arthritis individuals, independent of pre-treatment levels. This strategy has been recently applied to bone markers to predict the efficacy of bisphosphonates on fracture risk in osteoporosis[36] or skeletal related events in patients with cancer and bone metastases[37]. From a statistical standpoint, the use of a parameter (change from baseline) that uses two estimates of cartilage degradation in its calculation, each with its own variability is likely to be less likely to allow evaluation of a relationship. We thus dichotomized patients in low and high CTX-II levels at baseline and at 6 months using the mean levels of healthy non-arthritis individuals which have previously used to predict radiological progression in patients with RA[27]. We found that patients who started with low CTX-II levels and who remained at low levels after 6 months of treatment had a lower risk of progression than those who had high values at both time points. These data suggest that the maintenance of low CTX-II levels over 6 months may be an important factor to identify patients who are relatively protected from long-term radiological progression in knee OA. Indeed among these patients only 6% demonstrated a loss of JSW $\geq$ 0.6 mm after 24 months. Although risedronate treatment resulted in low CTX-II levels to a larger proportion than in patients receiving placebo, less than 30% of patients receiving the lowest doses of 5 mg/day and 35 mg/week had low values 6 months after initiating treatment. In the highest dose groups of 50 mg/week and 15 mg/day – which were the only regimens that had a significant effect on subchondral bone turnover[33] – this proportion increased but remained below or equal to 50%. This together with the limited progression observed in this study compared for example to the recent doxycycline trial[6] may explain the lack of apparent radiological effects of risedronate in that study, despite a significant, but probably insufficient, reduction of CTX-II. The validity of the CTX-II cut-off values used in that study to predict radiological progression will have, however, to be confirmed in other prospective studies of patients with knee OA.

Our study has certain strengths and some limitations. This is the largest study measuring bone and cartilage markers in a randomized controlled trial with any therapy in OA. Progression was assessed by a highly standardized radiographic technique using fluoroscopically positioned flexed knee view. Our study also has some limitations. As previously reported in this population[18], only a small

proportion of patients demonstrated significant radiological progression and contributes to the mean change of JSW. It was designed as a randomized trial to test the efficacy of risedronate on radiological progression, not to test the predictive value of CTX-II. We did not assess knee alignment and thus could not conduct an analysis of this parameter. We did not evaluate the status of other potential sources of CTX-II in the body, such as the hips, hands, spine which contribute to urinary levels of that marker[26]. We also did not measure other potentially useful biochemical markers of OA progression including the type II collagen degradation markers C2C, C12C[38], Helix-II[39] and Coll2-1[40], the type II collagen synthesis markers; C-terminal propeptide of type II procollagen (PIICP)-[38] and N-terminal propeptide of type IIA procollagen (PIIANP)-[13], cartilage oligomeric matrix protein[41] and hyaluronic acid[14]. However, urinary CTX-II has been shown to be associated with radiological OA progression in several prospective studies and it is currently the only systemic cartilage biochemical marker shown to decrease with bisphosphonates therapy.

Conclusion

This study has shown that measurements of urinary CTX-II prior to initiating treatment with risedronate and 6 months after the start of therapy provide useful information with respect to the radiographic prognosis of patients with knee OA, in combination with other known risk factors. In the context of future clinical development program for new SMOAD medicines, the CTX-II profile may be useful to judge the likelihood of long-term radiological progression and hence might help accelerate SMOAD development. Further evaluation of the utility of CTX-II and other biochemical markers of turnover of the joint tissues for clinical studies of potential SMOADs is recommended.

Conflict of interest

WSA, GAC, JFB and JMM are employee of P&G and own stocks or options in P&G. CC is CEO of Nordic Biosciences A/S which manufactures the assay for CTX-II. PG, SC and COB had received consultancies fees from P&G.

Author's contribution

PG planned the analyses of the data and wrote the manuscript. WSA, JFB and JMM planned the clinical trial. GAC performed the statistical analyses. PGC, SBC and COB recruited patients and assisted with the writing of the manuscript. CC performed CTX-II measurements. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to acknowledge the participation the following investigators: NA: (Canada) W Bensen, E Keystone, JP Pelletier and H Tannenbaum. (USA) RD Altman, HS Baraf, SB Herbert, JM Bathon, CO Bingham, JA Block, DG Borenstein, A Brodsky, SB Cohen, B Corser, T Cupps, JP Donohue, RD Emkey, J Fidelholtz, JZ Forstot, CM Franklin, KH Fye, N Gaylis, J Geohas, JS Gimbel, GV Gordon, R Gordon, TN Hangartner, BK Harris, R Katz, S Kolasinski, JA Markenson, M Neuwelt, C Radis, W Riskin, P Rosenthal, T Schnitzer, R Severance, Y Sherrer, J Tesser, C Thorne, N Wei and MA Weitz.

Europe: (Austria) J Smolen, H Broll. (Czech Republic) Z Chicy and K Pavelka. (France) P Bourgeois, A Kahan, J-M LeParc, T Bardin and M Dougados. (Germany) H Gaulrapp, V Jaegermann, H Rechl, M Talke, H Zippel, J Zacher and A Wagenitz. (Hungary) G Balint. (Ireland) B Bresnihan, E Casey, CJ McCarthy, MG Molloy and P O'Connell. (Italy) S Adami, M Cutolo, M Bevilacqua and L Sinigaglia. (Netherlands) JWJ Bijlsma, JD Moolenburgh and V Van de Walle. (Poland) E Czerwinski, Filipowicz-Sosnowska, P Gluszko, R Lorenc, J Przedlacki, A Sawicki, L Szczepanski and W Tlustochowicz. (Slovenia/Croatia) B Curkovic, A Pahor and B Rozman.

We thank Curtis Hayes and Cornelius van Kuijk for radiographic design and evaluations. We thank the investigators, the study coordinators and personnel at all investigative sites and radiographic facilities. We gratefully acknowledge the participation of the almost 2500 patients who were involved in these clinical trials. We would like to acknowledge the assistance of Ruby Xia for programming support.

References

1. Quam JP, Michet CJ Jr, Wilson MG, Rand JA, Ilstrup DM, Melton LJ 3rd, *et al*. Total knee arthroplasty: a population-based study. Mayo Clin Proc 1991;66:589–95.
2. Hochberg MC, Altman RD, Brand KD, Clark BM, Dieppe PA, Griffin MR, *et al*. Guidelines for the medical management of osteoarthritis. Part II. Osteoarthritis of the knee. Arthritis Rheum 1995;38:1541–6.
3. Reginster JY, Derosy R, Rovati LC, Lee RL, Lejeune E, Bruyerre O, *et al*. Long term effects of glucosamine suphate on osteoarthritis progression: a randomized, placebo-controlled clinical trial. Lancet 2001; 357:251–6.
4. Pavelka K, Gatterova J, Olejarova M, Machacek S, Giovanelli G, Rovati LC. Glucosamine sulphate use and delay of progression of knee osteoarthritis: a 3 year, randomized, placebo controlled double blind study. Arch Intern Med 2002;162:2113–23.
5. Dougados M, Nguyen M, Berdah L, Mazières B, Vignon E, Lequesne M. Evaluation of the structure-modifying effects of diacerein in hip osteoarthritis. Arthritis Rheum 2002;44:2539–47.
6. Brandt KD, Mazzuca SA, Katz BP, Lane KA, Buckwalter KA, Yocum DE, *et al*. Effects of doxycycline on progression of osteoarthritis: results of a randomized, placebo-controlled, double-blind trial. Arthritis Rheum 2005;52:2015–25.
7. Dieppe P, Cushnaghan J, Young P, Kirwan J. Prediction of the progression of joint space narrowing in osteoarthritis of the knee by bone scintigraphy. Ann Rheum Dis 1993;52:557–63.
8. Felson DT, McLaughlin S, Goggins J, LaValley MP, Gale ME, Totterman S, *et al*. Bone marrow edema and its relation to progression of knee osteoarthritis. Ann Intern Med 2003;139:330–6.
9. Hunter DJ, Zhang Y, Niu S, Goggins J, Amin S, LaValley MP, *et al*. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis Rheum 2006;54:1529–35.
10. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. Arthritis Rheum 2002;46: 3178–84.
11. Garnero P. Use of biochemical markers to study and follow patients with osteoarthritis. Curr Rheumatol Rep 2006;8:37–44.
12. Poole AR. Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. Rheum Dis Clin North Am 2003;29:803–18.
13. Garnero P, Ayral X, Rousseau JC, Christgau S, Sandell LJ, Dougados M, *et al*. Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. Arthritis Rheum 2002;46:2613–24.
14. Reijman M, Hazes JM, Bierma-Zienstra MA, Koes W, Christgau S, Christiansen C, *et al*. Predictive value of a cartilage degradation marker for radiologic osteoarthritis: the Rotterdam study. Arthritis Rheum 2004;50:2471–8.
15. Mazieres B, Garnero P, Gueguen A, Abbal M, Berdah L, Freiburghaus C, *et al*. Molecular markers of cartilage breakdown and synovitis are strong independent predictors of structural progression of hip osteoarthritis (OA). The ECHODIAH cohort. Ann Rheum Dis 2006;65:354–9.
16. Lehmann HJ, Mouritzen U, Christgau S, Cloos PA, Christiansen C. Effect of bisphosphonates on cartilage turnover assessed with a newly developed assay for collagen type II degradation products. Ann Rheum Dis 2002;61:530–3.

17. Spector TD, Conaghan PG, Buckland-Wright JC, Garnero P, Cline GA, Beary JF, *et al*. Effect of risedronate on joint structure and symptoms of knee osteoarthritis: results of the BRISK randomized, controlled trial. Arthritis Res Ther 2005;7:R625–33.
18. Bingham CO III, Buckland-Wright JC, Garnero P, Cohen SP, Dougados M, Silvano Adami S, *et al*. Risedronate decreases biochemical markers of cartilage degradation but does not decrease symptoms or slow radiographic progression in patients with medial compartment osteoarthritis of the knee: results of the two-year multinational knee osteoarthritis structural arthritis study. Arthritis Rheum 2006;54:3494–507.
19. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al*. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29:1039–49.
20. Buckland-Wright JC. Protocols for precise radio-anatomical positioning of the tibiofemoral and patellofemoral compartments of the knee. Osteoarthritis Cartilage 1995;(Suppl A):71–80.
21. Buckland-Wright JC, Bird CF, Ritter-Hrncirik CA, Cline GA, Tonkin C, Hangartner TN, *et al*. X-ray technologists' reproducibility from automated measurements of the medial tibiofemoral joint space width in knee osteoarthritis for a multicenter, multinational clinical trial. J Rheumatol 2003;30:329–38.
22. Hanson DA, Weis MA, Bollen AM, Maslan SL, Singer FR, Eyre DR. A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-telopeptides in urine. J Bone Miner Res 1992;7:1251–8.
23. Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, Gineyts E, *et al*. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone 2001;29:209–15.
24. Hahn GJ, Meeker WQ. Statistical Intervals: A Guide for Practitioners. New York: John Wiley & Sons, Inc; 1991 (Part 5.2).
25. Mouritzen U, Christgau S, Lehman HJ, Tanko LB, Christiansen C. Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index. Ann Rheum Dis 2003;62:332–6.
26. Garnero P, Sornay-Rendu E, Arlot M, Christiansen C, Delmas PD. Association between spine disc degeneration and type II Collagen degradation in postmenopausal women: the OFELY study. Arthritis Rheum 2004;50:3137–44.
27. Landewé R, Geusens P, Maarten B, Van der Heijde D, Lems W, Koppele JT, *et al*. Markers for type II collagen breakdown predict the effect of disease modifying treatment on long-term radiographic progression in patients with rheumatoid arthritis. Arthritis Rheum 2004; 50:1390–9.
28. Sondergaard BC, Henriksen K, Wulf H, Oestergaard S, Schurigt U, Brauer R, *et al*. Relative contribution of matrix metalloprotease and cysteine protease activities to cytokine-stimulated articular cartilage degradation. Osteoarthritis Cartilage 2006;4:738–48.
29. Garnero P, Christgau S, Delmas PD. The bisphosphonate zoledronate decreases type II collagen breakdown in patients with Paget's disease of bone. Bone 2001;28:461–4.
30. Oestergaard S, Sondergaard BC, Hoegh-Andersen P, Henriksen K, Qvist P, Christiansen C, *et al*. Effects of ovariectomy and estrogen therapy on type II collagen degradation and structural integrity of articular cartilage in rats. Arthritis Rheum 2006;54:2441–51.
31. Bay-Jensen A-C, Thomas AL, Ben-Tabassi N, Kristensen PW, Kjaersgaard-Andersen P, Sandell L, *et al*. Biochemical markers of type II collagen breakdown and synthesis are positioned at specific sites in human osteoarthritic knee cartilage. Osteoarthritis Cartilage 2008;16:615–23.
32. Meyer J FR, Prenger MC. Risedronate but not alendronate slows disease progression in the guinea pig model of primary osteoarthritis. J Bone Miner Res 2001;16(Suppl 1):SA472.
33. Buckland-Wright JC, Messent EA, Bingham CO III, Ward RJ, Tonkin C. A 2 yr longitudinal radiographic study examining the effect of a bisphosphonates (risedronate) upon subchondral bone loss in osteoarthritic knee patients. Rheumatology (Oxford) 2007; 46:257–64.
34. Konttinen YT, Salo T, Hanemaaijer R, Valleala H, Sorsa T, Sutinen M, *et al*. Collagenase-3 (MMP-13) and its activator in rheumatoid arthritis: localization in the pannus-hard tissue junction and inhibition by alendronate. Matrix Biol 1999;18:401–12.
35. Derenne S, Amiot M, Barillé S, Collette M, Robillard N, Berthaud P, *et al*. Zoledronate is a potent inhibitor of myeloma cell growth and secretion of Il-6 and MMP-1 by tumoral environment. J Bone Miner Res 1999; 14:2048–56.
36. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD. Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res 2003;18:1051–6.
37. Coleman RE, Major P, Lipton A, Brown JE, Lee KA, Smith M, *et al*. Predictive value of bone resorption and formation markers in cancer patients with bone metastases receiving the bisphosphonate zoledronic acid. J Clin Oncol 2005;23:4925–35.
38. Cahue S, Sharma L, Dunlop D, Ionescu M, Song J, Lobanok T, *et al*. The ratio of type II collagen breakdown to synthesis and its relationship with the progression of knee osteoarthritis. Osteoarthritis Cartilage 2007;15:819–23.
39. Garnero P, Charni N, Juillet F, Conrozier T, Vignon E. Increased urinary type II collagen helical (Helix-II) and C-telopeptide (CTX-II) levels are independently associated with a rapidly destructive hip osteoarthritis. Ann Rheum Dis 2006;65:1639–44.
40. Deberg MA, Labasse AH, Collette J, Seidel L, Reginster JY, Henrotin YE. One-year increase of Coll 2-1, a new marker of type II collagen degradation, in urine is highly predictive of radiological OA progression. Osteoarthritis Cartilage 2005;13:1059–65.
41. Sharif M, Kirwan JR, Elson CJ, Granell R, Clarke S. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. Arthritis Rheum 2004;50:2479–88.